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Notes on Bacillus Coli Communis and Related Forms:

Together with some Suggestions Concerning the Bacteriological Examination of Drinking-water.

(*From the pathological laboratory of the Bureau of Animal Industry of the U. S. Department of Agriculture.*)

BY

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NOTES ON BACILLUS COLI COMMUNIS AND RELATED FORMS,

TOGETHER WITH SOME SUGGESTIONS CONCERNING THE BACTERIOLOGICAL EXAMINATION OF DRINKING-WATER.

(*From the pathological laboratory of the Bureau of Animal Industry of the U. S. Department of Agriculture.*)

By Theobald Smith, M.D.

In the following pages are brought together a number of forms belonging to the species *B. coli communis*, as well as a variety of closely related forms which have been the object of so much attention and discussion during the past few years. They have been studied as carefully as time permitted and certain general relationships outlined which may be of service to future systematists. Attention has been specially directed toward their behavior in fermentation-tubes containing sugar-bouillon. The sharp distinctions brought out by this procedure between many of these forms on the one hand and *B. coli* and *B. typhosus* on the other are, to say the least, a relief from the uncertainty of the usual potato-culture and the variable intensity of the indol-reaction. These distinctions are emphasized in the following pages not for the purpose of making them the sole basis of classification, but to call attention to the necessity of applying the fermentation-test more accurately than has been done hitherto. A careful perusal of these pages will, I believe, convince the reader that the ordinary means of determining the production of gas are all but useless in the differentiation of related forms.

In several former publications[1] the writer has pointed out the value of the reaction of bacteria in the presence of carbohydrates as a means of differentiation. This is now generally conceded. All that is needed is to apply this test more precisely. In the fermentation-tube, when carbohydrates are present, the diagnostic characters exhibited by bacteria are not exhausted by the production of gas. There should always be taken into consideration:

1. The evolution of gas as to (*a*) total quantity, (*b*) rate of accumulation, (*c*) approximate composition, (*d*) temperature most favorable.
2. Formation of acids.
3. Behavior toward different carbohydrates.

This scheme indicates on the surface a large amount of work. As a matter of fact, there is little more work involved than in the exhaustive study of an ordinary bouillon-culture. The handling of the tube itself requires a little more care, but if the various minor suggestions laid down

in the article above referred to are heeded, no difficulty will be experienced.

The writer is fully aware of the fact that since his first publication on the use of the fermentation-tube much has been done in determining more fundamentally the changes induced in carbohydrates by bacteria.* The chemical work involved is too extensive, however, for the rapid differentiation of species and varieties. The fermentation-tube furnishes unequivocal characters promptly while permitting the comparative simultaneous study of a series of cultures with little trouble.

In the application of the test with bouillon containing carbohydrates other than the most easily attacked glucose or dextrose a difficulty arises which hampers us to a certain extent. Most beef (and the flesh of other animals as well) contains a fermentescible substance acting like dextrose. In testing with dextrose this does not interfere. When, however, other sugars are to be employed only that bouillon should be used in which gas does not appear during the multiplication of some gas-producing bacterium. The dextrose-free beef I have encountered in about one-fifth of the beef purchased. Recently I have found the flesh from cattle in advanced stages of tuberculosis, those that show signs of emaciation, free from sugar. Such bouillon should be set aside and used only with saccharose, lactose, or other sugars, excluding dextrose. Beef placed in boiling water immediately after slaughter has also been found free from fermentescible substances.

The fermentation-tests referred to in this article were all made at the temperature of 36°–37° C., unless it is otherwise stated. This is important, as the amount of gas produced varies with the temperature. This may be most abundant at 37° C., or at 20°–25° C., according to the species under observation. With *B. coli* the higher temperature is the most favorable. With some related forms it is the lower temperature. This is illustrated by some observations recorded in the tables.

The use of the fermentation-tube may be briefly summarized in the following steps:

1. The preparation of peptonized beef-broth in the ordinary way.
2. The preliminary test for sugar in two or three fermentation-tubes with *B. cloacæ*.†
3. If found producing gas, it may be used for dextrose-, but not for saccharose- or lactose-bouillon. Should other bouillon be not at hand, it may be used with other sugars, provided the species to be studied is tested in the bouillon containing no added sugar and the quantity of gas produced carefully noted and deducted from the total gas produced after sugars have been added.
4. The sugars should be added in the proportion of one per cent.

* For some of the literature on this subject see (1).

† See table on pages 16 *et seq.* Nos. 34, 35, and 36.

THE COLON-GROUP.

The importance of *B. coli communis* in pathology has been insisted upon for some years past. The frequent isolation of colon-bacilli from the diseased human and animal body is sufficient to account for this attitude. It has been claimed to be the cause of typhoid fever, diarrhœal diseases of infants and adults, various affections of the urinary tract, and peritonitis. In animals it has been regarded as the cause of abortion in cows, Texas fever, septicæmia in fowls, and has been confounded with the hog-cholera group of bacilli.

Whether the colon-bacillus is or is not the cause of some of the lesions with which it has been found associated is not to be answered at the present time, nor does it require an answer or even a discussion in this place. It is sufficient to note that it frequently penetrates into the body during life to induce us to treat the group with a certain dignity which it does not deserve otherwise.

Its relation to hygiene is defined by its frequent presence in water. For a number of years the writer has regarded the presence of *B. coli* in water as a valuable indication or symptom of pollution. This indicator has been looked upon by some as of little value. How far the one or the other position shall prove to be the acceptable one future work must decide.* Arguments for or against the significance of the colon-group both in the body and in water can only be properly dealt with when all shall have agreed upon what is to be called *B. coli communis* and when the related forms shall have been assigned to their proper place. It is one of the main objects of this paper to suggest a standard and to stimulate further research into intestinal bacteria in accordance with this standard until we know definitely what species or varieties are true inhabitants of the digestive tract and what species are there temporarily and really belong elsewhere.

The distinguishing characters of *B. coli* have been presented by different writers in different ways. Having been studied mainly in comparison with the typhoid bacillus, its characters have been defined with the latter as a standard. Other things being equal, *B. coli*, according to Escherich, produces gas in solutions of sugar and coagulates milk. C. Fränkel considered it non-motile. Chantemesse and Widal looked upon the gas-production in lactose-bouillon as diagnostic. Kitasato regarded the indol-reaction as an important differential character. It would be going wholly beyond the scope of this paper to discuss all the writings upon the colon-group. Only those can be noticed which bear directly upon the subject of the paper.

* The fact that *B. coli* is an even more prolific inhabitant of the intestines of certain domestic animals (determined by the writer in a series of unpublished studies in 1889 and 1890, and since then by others[2]) does not destroy the value of *B. coli* as an indicator of fecal contamination, for hygiene has no justification whatever for considering the intestinal contents of the higher mammals and birds as entirely harmless to man.

Achard and Renault,[3] in endeavoring to differentiate bacteria of this group, obtained from the urinary tract, tried the method of sowing one form upon a substratum which had previously served for the growth of another form. If development took place, the forms were regarded as belonging to different types. By this procedure five types were found. These types varied furthermore in their behavior in lactose-bouillon and milk and toward the indol-test.

The criterion thus established by these authors seems scarcely to deserve the importance attached to it. That it may serve as one of the means of differentiation cannot be gainsaid. Yet it should be borne in mind that a slightly increased sensitiveness to acids or alkalies may be at the bottom of this criterion, a sensitiveness which may be variable among the varieties of the same pathogenic species.[10]

Gilbert and Leon,[4] in endeavoring to group colon-bacilli, paid special attention to their motility, their behavior in lactose-bouillon and in milk, and toward the indol-reaction. They discuss quite thoroughly the application of these tests, and point out gradations in the intensity of the indol-reaction. They fail, however, with all other observers, to perceive that colon-bacilli are all active when examined from young surface-colonies in gelatine. They conclude by describing the type colon-bacillus as a motile, non-liquefying bacillus, giving a rich yellow growth on potato, fermenting lactose-bouillon, coagulating milk, and giving the indol-reaction.

This definition covers the ground very well, and includes the two varieties—one fermenting saccharose, the other not, as defined by the writer in the following pages. It should be stated, furthermore, that with some varieties of potato no growth may appear in the thermostat. Again, the fermentation-reaction in lactose-bouillon is inadequate, and the definition of the species as given by these writers is saved from total wreckage by the addition of the positive indol-reaction, as may be seen by an examination of the tables given below.

Perhaps the most exhaustive study of bacilli resembling *B. typhosus* and the colon-group was made by Germano and Maurea[5] in Kruse's laboratory. These authors made use of a variety of tests, including the observation of motility, behavior in jequirity solution, litmus whey, production of alkali in peptone-bouillon, reducing action, the Gram stain and the gas-production in 2 per cent. glucose-, saccharose-, and lactose-agar. This painstaking work, in which 100 cultures are submitted to these tests, does not aid us in defining the different varieties of the colon-group, but simply in distinguishing them from the typhoid bacillus. In the published list there are fifteen forms (several are cultures of *B. typhosus*) producing no gas in any sugar, thirty producing gas in three sugars, nineteen producing gas in glucose- and lactose-agar, and one producing gas only in glucose-agar. The rest are characterized indefinitely

as producing a little or a moderate quantity of gas in one or the other of the agar-tubes. This might be traced to muscle-sugar in some cases in which bubbles appeared in saccharose- and lactose-agar. The total quantity of gas produced and the relative amount of CO_2 are, of course, not determinable in agar. An important character, namely, the reaction of the sugar-agar when gas is not formed, is not given. This important work is not criticised for what is determined in it, but only for what might have been found out with little extra trouble concerning the colon-group.

Fremlin[6] in comparing colon-bacilli from different sources employs glucose-bouillon only. The motility is also not well defined.

Ury[7] examined *B. coli* and the bacillus of Friedländer, and comes to the rather antiquated conclusion that they are different.

Beckmann[8] finds in ground-water not exposed to pollution typhoid-like bacilli which ferment glucose and coagulate milk after a variable period. These are called *B. coli*. They might as well be ranged under *B. cloacæ* so far as the meagre description goes.

In order to establish a type-species of *B. coli communis* I would suggest that those forms be regarded as true to this species which grow on gelatine in the form of delicate bluish or more opaque whitish expansions with irregular margin, which are actively motile when examined in the hanging drop from young surface-colonies taken from gelatine plates, which coagulate milk within a few days; grow upon potato either as a rich-pale or brownish-yellow deposit, or merely as a glistening, barely recognizable layer, and which give a distinct indol-reaction. Their behavior in the fermentation-tube must conform to the following scheme:

Variety *α*. One per cent. dextrose-bouillon (at 37° C.).

Total gas, approximately 1/2; H/CO_2 approximately 2/1; reaction strongly acid.

One per cent. lactose-bouillon:

As in dextrose-bouillon (with slight variation.)

One per cent. saccharose-bouillon:

Gas-production slower than in the preceding, lasting from seven to fourteen days. Total gas finally about 2/3; H/CO_2 nearly 3/2. The final reaction in the bulb may be slightly acid or alkaline, according to the rate of gas-production.

Variety *β*. The same in all respects excepting as to its behavior in saccharose-bouillon. Neither gas nor acids* are formed in it.

In the table *B. coli α* and *β* from a variety of sources are represented. One of them (No. 14) did not give the indol-reaction, and is therefore, strictly speaking, to be ranged under another variety. Besides these a

* Throughout this publication an acid reaction signifies the distinct reddening of litmus-paper not changed by the drying of the paper or the boiling of the fluid. The reaction of the open bulb of the fermentation-tube is referred to.

number of other provisional groups are included in the table, which, with the exception of the *B. lactis aërogenes* group, might be regarded as belonging either to the colon-group or to the typhoid group. The colonies on gelatine are all of the coli type, and all are actively motile forms of about the same size. They were included in the table because the ordinary microscopic and cultural characters failed to distinguish them from these two types. Before referring briefly to the salient features of these intermediate forms, it is desirable to point out once more briefly the fundamental difference between *B. coli* on the one hand and the large hog-cholera group (including *B. enteriditis* and *B. typhi murium*) and the typhoid bacillus on the other. The fermentation-reaction of the hog-cholera group (16a of the table) is as follows:

One per cent. dextrose-bouillon: total gas 1/2; H/CO_2 2/1;* strongly acid reaction; 1 per cent. lactose-bouillon: no gas or acids; 1 per cent. saccharose-bouillon: no gas or acids.

The hog-cholera bacillus, while identical with *B. coli* in its behavior in dextrose-bouillon, differs wholly from the latter in having no effect on lactose (and milk) and saccharose.

The typhoid bacillus (28a) differs from both:

One per cent. dextrose-bouillon: no gas, markedly acid reaction;† 1 per cent. lactose-bouillon: no gas, no acid reaction;‡ 1 per cent. saccharose-bouillon: no gas, no acid reaction.

The use of lactose-bouillon alone is not sufficient as a distinguishing character between typhoid and pseudo-typhoid forms, for the whole hog-cholera group and many other forms fail to act upon lactose.

BACILLI RESEMBLING B. COLI COMMUNIS.

A species which is of considerable interest is No. 16:

Bacilli morphologically like *B. coli*, in active motion when taken from fresh gelatine surface-colonies and examined in water. On potato a yellowish deposit or none, according to the potato used. Milk coagulated in about eight days. The reactions in sugar-bouillon are as follows:

Dextrose-bouillon, identical with *B. coli.*

Saccharose-bouillon, no action.

Lactose-bouillon, *no evolution of gas, but markedly acid reaction.*

This species thus lacks the power to evolve gas in lactose-bouillon,

* The figures are throughout this article to be regarded as merely approximate. Slight variations in the quantity of gas produced may occur in parallel cultures of the same organism.

† The action of this bacillus on dextrose is usually not recognized because gas is not formed. As pointed out by Perié[9], it forms acids from dextrose sufficient to coagulate milk. Anyone may try this by adding some sterile dextrose solution to milk and inoculating with the typhoid bacillus. Within a week the milk will be solid.

‡ In a former article (*Centralblatt f. Bacteriologie*, xi. 367) I stated that the reaction of saccharose- and lactose-bouillon inoculated with typhoid bacilli is transiently acid. This is an error due to the use of bouillon not free from muscle-sugar. A transient acidity in such bouillon is common to a large number of bacteria.

though it still retains the power to form acids from the sugar, and hence to coagulate milk.

A slightly aberrant group of apparently wide distribution, as I have frequently encountered it in well-water, is the following (Nos. 17, 18, and 19):

Bacilli in form, size, and motility like *B. coli.* Surface-colonies on gelatine plates differ slightly from those of *B. coli* (but not invariably) in the earliest stages of growth. They are roundish, slightly convex patches, with peculiar radial, linear markings on the disk, which now and then remind one of hair combed from a central point outward. These markings may disappear partly or wholly under artificial cultivation. In the later stages of growth these colonies expand into irregular patches not distinguishable from those of *B. coli.* Milk is coagulated in four or five days. On potato growth may be yellowish or not recognizable (tested but once). The fermentation-reaction is peculiar:

Dextrose-bouillon: no evolution of gas; reaction acid.

Saccharose-bouillon; no evolution of gas; reaction unchanged.

Lactose-bouillon: gas approximately 1/2; H/CO_2, 2/1 or 3/1.

This species has thus no power to produce gas in dextrose-bouillon, and in this respect resembles the typhoid bacillus. It fails to act upon saccharose. In lactose-bouillon its behavior is practically like that of *B. coli.* To this statement one exception must be made. The gas appears quite tardily. The earliest bubble does not appear for several days, and may be delayed nearly a week. With *B. coli* most of the gas appears within twenty-four hours, and all within three or four days. In one of these forms, the only one tried, multiplication on potato appears to be more vigorous at 20° to 25° than at 37° C. Fermentation-tubes should be kept at least a week before they are rejected in order that the retarded appearance of gas may not be overlooked.

This species would come under the designation of *B. coli* if judged according to standards hitherto employed. It coagulates milk and produces gas in lactose-bouillon, the fluid chosen by Chantemesse and Widal. But besides its different behavior toward dextrose it does not produce indol. Its occurrence as an intestinal form has not been demonstrated, nor can it be correlated with forms already described until the gas-test is more thoroughly applied.

TRANSITIONAL FORMS.

In the table a number of cultures are thus designated, because they stand near to those forms already described in their behavior toward sugars, or else because they approach the pseudo-typhoid and the true typhoid bacilli in producing either a very little or no gas.

Thus, No. 20 is morphologically like *B. coli,* but its colonies have the markings of the preceding group in the earliest stages. Milk is coagu-

lated in five days. On potato an abundant straw-colored layer is formed. In dextrose-bouillon it produces only a little gas at 37° C., and becomes distinctly acid. Upon saccharose it has no effect. In lactose-bouillon the reaction becomes strongly acid. When the fermentation-tubes are kept in the laboratory temperature the gas formula changes materially. This suggests that a lower temperature is the more suitable for this species.

No. 21 is also a peculiar species as regards the gas-test. The gas formulæ at 37° C. and at 20° to 25° C. are quite different from each other.

No. 22 stands very close to the hog-cholera group. The behavior in lactose-bouillon, in which a little gas is set free, is significant as marking the dividing-line, for I have never observed even a trace of gas in the hog-cholera group. It will be noticed that the action on milk-sugar is too feeble to produce any change in milk.

No. 23 is identical with the group described above (Nos. 17–19) in producing gas only in lactose-bouillon. It acts upon the other sugars also in producing acids. While it resembles these forms in some characters it differs from them in others (non-motility, fleshy form of colony) and approaches the lactis aërogenes group.

Nos. 24 and 25 form a distinct group as regards the gas-reaction, but differ from each in their behavior in milk and toward the indol-test. The colonies resemble those of *B. coli* for a time. Later on, the margin of the expanded surface-colony becomes quite regularly scalloped.

PSEUDO-TYPHOID BACILLI.

These are naturally of much interest, as they resemble typhoid bacilli in not producing even a trace of gas in sugar-bouillon. Three of these were isolated (Nos. 26, 27, and 28), and these differ from each other in certain characters.

No. 27 resembles *B. coli* in the form, size, and motility of the bacilli. On gelatine-plates the surface-colonies expand into patches of irregular outline. In the earlier stages of growth both deep and surface colonies may have strands of wavy lines running radially across the disk. These are not constant, however. Bouillon becomes quite turbid. Milk is not changed after one or more weeks in the thermostat, but is coagulated when boiled.

On potato the growth varies. In the thermostat no growth appears. In the temperature of the laboratory, however, an abundant, colorless, glistening growth appeared after some days, distinguished from the now rosy-colored potato only by the relief and the glistening. Gas is not evolved. The reaction in dextrose-bouillon is pronouncedly acid, in lactose-bouillon feebly so, and in saccharose-bouillon alkaline.

No. 26 differs from the preceding form in some respects. The colonies have no characteristic markings; *i. e.*, they resemble *B. coli* closely. The

growth on potato is yellowish or cream-colored, and the reaction of saccharose-bouillon acid.

No. 28 stands nearest to the true typhoid bacillus. It was isolated from a city well, the bacteriological examination of which did not indicate much, if any, fecal contamination. The colonies of this form expand like those of *B. coli*. A visible growth on potato was not observed at 37° C. The behavior in bouillon containing the three sugars is identical with that of the true typhoid bacillus; *i. e* , there is no gas formed, and the reaction of the dextrose-bouillon only becomes acid. Milk-cultures remain unchanged even after boiling.

This form differs from the genuine typhoid bacillus in a more vigorous growth in gelatine and in a much less persistent motility in culture-fluids. In bouillon a cohesive condition of the deposit was noticed after one or more weeks. That this form is not the recognized typhoid bacillus may thus be conceded. However, it would be impossible to state that it may not be one of the varieties of this pathogenic species. The study of such varieties, if any exist, is, in spite of the work of Babes, still in its beginnings. If the writer were to use his experience gained in the study of a closely related group of pathogenic organisms, he should say that No. 28 could not be positively excluded from the genuine typhoid bacilli, since variations within the hog-cholera group itself have been found to be quite wide.[10]

B. LACTIS AËROGENES.

The failure on the part of many observers to note the motility of *B. coli* has led to the confusion of this group with the *B. lactis aërogenes* group. The latter is frequently referred to in bacteriological publications, and appears to hold the same enviable position with *B. coli* in the production of various forms of disease. This group should, therefore, be subdivided, and the various forms, brought together under it, defined more rigidly. That there is ample reason for such differentiation is shown below.

The cultures which I have regarded as coming under this group are non-motile bacilli, distinctly plumper and perhaps a trifle shorter than *B. coli*. In young cultures they are mere ovals. The surface-colonies on gelatine-plates are distinguished from those of *B. coli* in being fleshier, slightly convex, and having smooth, abrupt borders. They are, in other words, distinctly disk-shaped, and when allowed to expand fully in gelatine rolls protected from drying they frequently appear like lids provided with a central knob. The colony is not cohesive. The platinum needle readily breaks it up. This is due to the existence of a distinct capsule, or at least capsular substance usually recognizable on the border of the hanging drop by a regular spacing of contiguous bacilli. When roll-cultures stand upright the large colonies may flow

*

partly down the sides of the tube and raise the suspicion that the gelatine is being slowly liquefied, which is not the case.

These bacilli are all gas-producing, and the formulæ vary considerably.

Thus, for No. 29 the gas formulæ for dextrose- and lactose-bouillon are precisely like those for *B. coli*. Neither gas nor acids appear in saccharose-bouillon. On potato a rich, yellowish growth is formed. Milk is firmly coagulated in a few days. The indol-reaction is positive. Were it not for the morphology this bacillus would be ranged under *B. coli*.

A species represented by Nos. 31, 32, and 33 is quite different from the preceding in its physiological characters. It ferments the three sugars and starch. On potato there is formed an exceedingly rich pale-yellow or ashen growth, not cohesive. The potato becomes much discolored, and gas bubbles may appear. Milk is coagulated promptly in twenty-four to forty-eight hours. The gas-production differs from that of any of the species described. In dextrose-bouillon the total gas is 5/6 to 6/6; *i. e.*, up to 100 per cent. The ratio H/CO_2 is nearly unity, the volume of hydrogen slightly predominating. The acid reaction is feebler than in the colon-group. When the fermentation is somewhat retarded the open bulb may be alkaline after the close of the fermentation. When gas collects more rapidly the reaction does not become alkaline. The gas-reaction for saccharose and lactose is the same as that for dextrose. The gas-reactions for starch have not been studied.

A slightly aberrant form (No. 30) was found in spring-water, and perhaps derived from the manure of animals frequenting it. The potato-growth is identical with that of the preceding, but the coagulation of milk proceeds more slowly, not being completed for five or six days. The gas-reactions are likewise different, approaching those of the colon-group:

In dextrose-bouillon, total gas 1/3; H/CO_2 3/1; reaction acid.

In lactose-bouillon, total gas 11/12; H/CO_2 3/2; reaction acid.

In saccharose-bouillon, total gas 2/3; H/CO_2 2/1; reaction acid.

From these figures it will be seen that the total gas-production is less and the relative amount of hydrogen greater than in the preceding species. Here, again, is illustrated the fact frequently noted by the writer that where gas is evolved the intensity of the acid reaction is proportionate to the relative amount of hydrogen set free.

Forms similar to the members of the group as described above were encountered in the intestine of the pig, and have been compared among themselves and with B. Friedländer in a former publication.[11] * The same variations are there noted. The gas-reactions as given are not

* Denys and Martin have made similar comparative studies recently: Sur le rapport du pneumo-bacille de Friedländer, du ferment lactique et de quelques autres organismes avec le *B. lactis aërogenes* et le *B. typhosus*. La cellule ix. (1893) p. 261. (The original not obtainable by the writer.)

strictly correct, however, as the bouillon used was not free from muscle-sugar.

A peculiar form already referred to is represented by No. 23 of the table. It possesses the morphological character of the lactis aërogenes group in being short, plump, and non-motile, and provided with a capsule or at least capsular (zooglœar) substance, recognizable in masses of bacteria from a colony. The latter is fleshy, round, grayish-white, and not viscid. Milk is coagulated in two or three days. On potato its growth remains invisible. It produces gas in lactose, but not in glucose or saccharose bouillon. These fluids are made distinctly acid, however. The type of gas-production in lactose-bouillon is like that of *B. coli*.

Before concluding this portion of the work it is desirable to call attention to a species very frequently encountered, by the processes given below, in surface- and well-water polluted as well as unpolluted. This species is represented by Nos. 34, 35, and 36 of the table. These bacilli are perhaps a trifle smaller than *B. coli*, and more nearly resemble the hog-cholera group. Their motility is more persistent than that of *B. coli*. They differ from the latter in some important characters. They slowly liquefy gelatine, the gas-reaction is wholly different, and indol is not produced. Nevertheless, they may be mistaken for *B. coli* in hurried determinations, because the liquefaction on plates owing to its retarded appearance may be entirely overlooked if the plates are allowed to dry out rapidly. Even in gelatine-tubes the liquefaction may not appear in one or two weeks. If, moreover, agar be used with the various sugars in place of the fermentation-tube the peculiar gas formula will not be recognized, and the chances of error in diagnosis increased. From the table it will be noticed that in dextrose- and saccharose-bouillon the gas drives out the fluid almost entirely, and the CO_2 always predominates over the hydrogen. The gas formula in lactose-bouillon is variable, owing to the slow accumulation of gas. V. A. Moore[12] has found this species (or group) frequenting rusty spots on Indian corn. This fact strengthens the hypothesis held by the writer that the habitat of this species is vegetation, living and dead.

A few words may be added here concerning the correlation of certain culture-tests, such as that of lactose-bouillon and milk. It may be laid down as a general rule that if acids are produced in the former the latter will be coagulated either spontaneously or after the application of heat. Both tests are necessary to supplement each other. The lactose-bouillon informs us whether any acids are produced, the milk the intensity of acid formation. This may vary somewhat even for the same culture, and the production of acids in milk may be overlooked unless lactose-bouillon be used at the same time. In a recent study of a mouse septicæmia (or swine erysipelas) culture the writer was led to assume that this culture might coagulate milk because it invariably produced

a pronouncedly acid reaction of lactose-bouillon. Several tests in milk showed that when the cultures were boiled they might or might not become coagulated. This variability may depend on the initial acidity of the milk or upon the vigor of the culture used.

The variable behavior of potatoes as culture-media has been the subject of such frequent comment that I may well pass it by, noting simply the fact that the potato may be utilized to test the action of bacteria on starch. The permanence of the acid reaction (especially of the water in the tube) may at least for the present be regarded as a sign that starch is being split up. If the potato-cylinder fits snugly into the test-tube and dips into the water, any gas bubbles formed will become imprisoned between the potato and the sides of the tube. The cultures No. 31, 32, and 33 are evidently gas-producers in the presence of starch. This phenomenon may be witnessed by adding starch to glucose-free bouillon in the fermentation-tube before final sterilization. Gas-production from starch is also a property of the *B. cloacæ* group.

The brief sketch which I have given of a variety of forms which may be mistaken for *B. coli* shows that the fermentation-test must be applied at least as fully as I have used it in order that the colon-bacillus may gain more relief and distinctness. It is evident that the simple determination of gas-production in sugar-agar is wholly insufficient owing to the great variety of gas-producing bacteria. It is furthermore clear that neither lactose nor glucose bouillon by themselves define anything, and that at least the three kinds of sugar-bouillon must be used together. For the more accurate subdivision of saprophytes it will be necessary to extend the test to starch, and, perhaps, other carbohydrates, as has been done more especially by Frankland and those working under Duclaux.

The objection occasionally urged that bacteria lose the power of gas-production during artificial cultivation I have already met in a former publication[1] with convincing illustrations. At this date I have no reason to change the views expressed elsewhere that gas and acid-production are too fundamental characters to be lost. The effect of cultivation on gas-production for some groups is *nil*, for others the rate and quantity may suffer somewhat, but the production does not disappear. For a small number (Nos. 24 and 25 of the table), in which the amount of gas set free, when the bacteria were first isolated, was very small, the gas may or may not appear subsequently, according to partially known conditions, one of which is temperature. Such forms are probably transitional, whose acid-formula should be carefully studied. The amount of gas set free may vary slightly with the construction of the fermentation-tube, and the condition of the culture from which the tubes are inoculated. If this be old, partially dried out, there may be considerable variation in the total amount of gas. If, on the other hand, only fresh cultures are used, the variation from tube to tube is very slight. This may

account for the statements now and then made of the inconstancy of gas-production, statements which may probably be refuted by one passage of the culture through fresh media. In all these cases of temporary variation the formula H/CO_2 remains remarkably constant.

Besides the production or non-production of gases the appearance or non-appearance of acids in bouillon containing sugars merits our attention as of equal importance. Bacteria may produce acids with or without the evolution of gas, or they may leave the sugar untouched, in which case the reaction remains alkaline. The acid may be produced so slowly that it is masked by the alkaline tendency of bacteria in active multiplication. To this phase of the subject the writer hopes to return at some future date. It is in general sufficient to take the reaction of the fluid in the open bulb, as well as in the closed branch, before the tube is rejected, to cover all cases. In the table only the reaction of the open bulb is given.

The behavior of bacteria toward sugars in nutritive media is most probably a slow physiological adaptation to environment which affects a great variety of morphologically distinct forms and brings them nearer together. Hence it may be a guide to habitat and function rather than to descent. It is this adaptation which has probably brought members of the lactis aërogenes group so close to those of the colon-group that they have been considered as identical.* For the purposes of bacteriology, as applied to pathological and hygienic problems, it seems that the grouping according to function and bio-chemical activity is of most importance.

THE ISOLATION OF B. COLI COMMUNIS FROM WATER.

It has been the not unreasonable hope of bacteriology to find processes capable of revealing specific disease germs in water. While this problem may be regarded as solved in case of the spirillum of Asiatic cholera, it is far from solution in case of typhoid bacilli. Realizing for some years past the difficulties inherent in this problem on account of the presence of the colon-group in polluted water, I turned my attention to the latter as of possible use as an indicator of pollution. But even the detection of *B. coli* in ordinary gelatine-plates made from a small quantity of water is, as a rule, not possible, for the more contaminated a given water the richer the bacterial flora, which grows more rapidly than *B. coli* and soon liquefies the gelatine. Other special devices must therefore be brought into requisition. These are in the main the outcome of studies designed to reveal the presence of typhoid bacilli in water, and hence a brief reference to the latter will not be out of place.

Vincent[13] claims to have succeeded in finding typhoid bacilli, as well as colon-bacilli, by adding water to carbolized bouillon and cultivating

* See No. 29 of the table.

the mixture at 42° C. Several passages may be necessary. Foote[14] recommends agar-plates in which most bacteria are suppressed at 37° C. Wurtz[15] recommends agar to which lactose has been added, and which has been tinged with litmus. The colonies upon such a plate divide themselves into two great groups, those which act upon lactose, produce acids from it, and change the color of the colony to red, and those which fail to act upon lactose, and hence leave the blue color unchanged. The colon-bacilli will be found among the former, the typhoid bacilli among the latter. The great utility of this simple process has been strongly urged by Mathews[16] when the problem is to search for typhoid bacilli. Its usefulness in the quantitative estimation of fecal bacteria will be better known after the nature of the red colonies has been more exhaustively studied.

The difficulty of finding typhoid among colon-bacilli by the bouillon-method paves the way for the not unjustifiable suspicion that the frequently reported discovery of typhoid bacilli may have been in perhaps all cases the finding of pseudo-typhoid or colon-bacilli. This suspicion is voiced very recently by Grimbert,[17] who admits that it is impossible to find typhoid bacilli in the presence of the far outnumbering colon-bacilli. The writer some years ago found it impossible to isolate typhoid bacilli added directly to unsterilized water containing at the time about 300 bacteria in a c.cm.

The colon and related bacilli referred to in the table as coming from water were isolated according to two methods based upon that suggested by Rodet and G. Roux, and modified by Vincent. To flasks of bouillon containing about 100 c.cm. 50 c.cm. of water were added, the mixture quickly warmed and placed at 37° C. After sixteen to twenty-four hours gelatine-plates were made, and rarely more than one species found. When membranes appeared on the bouillon the flask was tilted and the fluid for the plates taken from the deeper layers. By this precaution *B. subtilis* and other liquefying bacilli were avoided. Carbolic acid was not added to the bouillon. The other similar process consisted in adding a small quantity of water to each one of a series of fermentation-tubes containing glucose bouillon and placing them in the thermostat.[18] The amount of water added depended on the probable amount of pollution, and varied from 0.1 to 1, or even more c.cm. If, after two or three days, any tube indicated the presence of *B. coli* by the quantity of gas formed, and the rate of evolution, plates were made from the deposit. Usually but one species, *B. coli*, appeared; sometimes two were found on the plates.

One objection to this method is the rapid destruction of bacteria in the strongly acid bouillon. Unless the isolation is attempted within a week, the fermentation-tube may be found sterile, or else some other spore-bearing bacillus taking the place of the colon-bacilli on the plate.

These two methods have been used, since 1891, by the writer with much satisfaction. The second has been adapted to the quantitative determination of the colon-bacilli. If, for example, 0.5 c.cm. of water be added to every one of a series of ten fermentation-tubes containing 1 per cent. dextrose, and if in two of these the colon-type of gas-production appears, we may safely conclude that there were two colon-bacilli in the 5 c.cm. used.

It may be urged that the presence of two or more gas-producing forms may mask the indications of the fermentation-tube. But gas-producing bacteria are not so numerous as a rule, and in exceptional cases a second test may be made with smaller quantities of water. A series of mixed infections made by inoculating the same tube with *B. coli* and some other gas-producing species has convinced the writer that in nearly all cases *B. coli* maintains its own type. This is probably due to the large amount of acid produced by *B. coli* in the dextrose-bouillon, which inhibits the growth of other species. The final strength of this acid appears to be equivalent, approximately, to 0.3 per cent. of normal acid, as determined by titration of potassium hydrate, when phenolphthalein is used as an indicator.

It will be noticed that stress is placed only upon the presence of *B. coli*. The widely distributed *B. cloacæ* is excluded, also those forms producing less gas than *B. coli*, among them *Proteus vulgaris*. This division may appear somewhat arbitrary; but it errs, if at all, on the conservative side. The gas-formulæ of regular inhabitants of the intestines other than those of *B. coli* are not yet studied, and until more is known of their relative abundance, as compared with *B. coli*, the ultimate value of the qualitative test cannot be estimated. Schardinger[19, 20] is inclined to look upon the presence of fermentative (*i. e.*, gas-producing) forms of all kinds in water as indicative of pollution. This is true if, by pollution, we mean filth of any kind. For the movement toward pollution, especially of surface-water, seems to begin with the appearance of gas-producers which steadily increase in numbers with the amount of pollution.

The wide distribution of the colon-group has led some writers to regard *B. coli* of little value as a symptom of fecal contamination. At present the tendency seems to be in the other direction, and more attention is being paid to processes designed to reveal its presence. Kleiber[21] in a recent article recommends the addition of 2 per cent. carbolic acid to the mixture of bouillon and water. Burri[22] adds 0.75 per cent. of dry sodium carbonate to the culture-fluids. The high temperature of the thermostat, coupled with the strong alkalinity, is designed to suppress all but colon-bacilli. Burri rightly suggests that the detection of fecal bacteria in a large quantity of water may lead to the condemnation of really good water. It is for the same reason that I regard quantitative methods of importance, and consider the inoculation of a series of tubes of more service than the addition of a large quantity of water to bouillon in a single flask.

	No.	Source.	Form of surface-colony on gelatine.	Motility	Gas-reaction in fermentation-tube containing one per cent. sugar-bouillon.									Potato.	Milk coagulated.	Indol-reaction.	Remarks.
					Dextrose.			Lactose.			Saccharose.						
					Total gas.	H CO_2	Reaction	Total gas.	H CO_2	Reaction	Total gas.	H CO_2	Reaction				
Bacillus coli communis.	1	River-water	Spreading	Motile	5/12	2/1	Acid	1/2	2/1+	Acid	1/2	2/1+	Acid	Yellowish	In 2 days	Positive	Nos. 1 to 8 inclusive produce gas in the saccharose.
	2	Human feces.	Spreading	Motile	1/2	2/1+	Acid	1/2	2/1	Acid	2/3	4/3	.....	Yellowish	In 2 days	Positive	Nos. 9 to 15 inclusive do not produce gas in saccharose.
	3	Human intestines.	Fleshier	Motile	1/2	2/1+	Acid	2/3	...	Acid	2/3	2/1+	Acid	.....	.....	.....	
	4	Liver of cow	Fleshier	Motile	7/12	2/1	Acid	7/12	2/1	Acid	5/6	6/5	Alk.	Yellowish	In 2–3 days	Positive	Cultures Nos. 4 and 9 were obtained, together with a streptococcus, from the liver of a case of parturient apoplexy.
	5	River-water	Spreading	Motile	1 * 2 1 3	2 1+ —	Acid	7 12 1 3	3 1+ —	Acid	7 12 1 3	3 2 —	Acid	Yellowish	In 1–2 days	Faint	
	6	Well-water	Spreading	Motile	1/2	3/1	Acid	2/3	2/1+	Acid	7/12	3/2	Alk.	No distinct growth	In 2–3 days	Positive	
	7	Human feces.	Spreading	Motile	1/2	2/1	Acid	1/2	2/1	Acid	2/3	4/3	.....	Yellowish	In 2 days	Positive	
	8	River-water	Spreading	Motile	1/2	2/1	Acid	1/2	2/1+	Acid	2/3	2/1+	Alk.	.....	.....	.....	* Where two sets of gas-reactions are given, the upper series refers to the temperature of 37° C., the lower to that of 20° to 25° C.
	9	Liver of cow	Spreading	Motile	1/2	9/5	Acid	7/12	5–/3	Acid	0	0	Alk.	Yellowish	In 2–3 days	Positive	
	10	River-water	Spreading	Motile	2/3	3/2	Acid	7/12	2/1	.....	0	0	Alk.	Yellowish	In 1–2 days	Positive	
	11	River-water	Fleshy	Motile	1/2	2+/1	Acid	1/2	2/1+	Acid	0	0	Alk.	Yellowish	In 2 days	Positive	
	12	River-water	Spreading	Motile	7/12	2/1+	Acid	1/2	2/1+	Acid	0	0	Alk.	No distinct growth	On boiling only	Positive	In a second test milk coagulates in four to five days.

Transitional forms.	13	River-water	Spreading	Motile	1 2	2 1	Acid	7 12	2 1—	Acid	0	0	Alk.	No distinct growth	In 2–3 days	Positive	Nos. 12 and 13 were isolated from the same sample. The surface-growth in gelatine stab-cultures of No. 12 became finely wrinkled and presented a ground-glass appearance, that of No 13 remained smooth.
	14	Well-water	Spreading	Motile	7 12	2 1	Acid	3 4	2 1—	Acid	bub'le	—	Alk.	No distinct growth	In 2–3 days	Negative	
	15	Cattle	Spreading	Motile	1 2	2 1	Acid	7 12	2 1	Acid	0	0	Alk.	.....	.....	Positive	
Transi-tional.	16	Well-water	Spreading	Motile	1 2	2 1	Acid	0 0	0 0	Acid	0	0	Alk.	Yellowish	In 8 days	Negative	
	16a	Hog-cholera	Small	Motile	1 2	2 1	Acid	0	0	Alk.	0	0	Alk.	Yellowish	Not coagulated even after boiling	Negative	
Pseudo-colon bacilli.	17	Well-water	Spreading	Motile	0	0	Acid	1 2 bub'le	2 1— —	Acid	0	0	Alk.	Yellowish	In 5 days	Negative	Potato-growth better at 20° C.
	18	Well-water	Spreading	Motile	0	0	Acid	5 12	3 1—	Acid	0	0	Acid	No distinct growth	In 5 days	.....	
	19	Well-water	Spreading	Motile	0	0	Acid	1 2	2 1—	Acid	0	0	Alk.	No distinct growth	In 4 days	Negative	
Transitional forms.	20	Well-water	Fleshier	Motile	1* 20 5 6	— 1 1	Acid	0 1 20	— —	Acid	0 0	0 0	Alk.	Yellowish	In 5 days	Negative	Potato-culture made a year later shows whitish growth.
	21	Well-water	Spreading	Motile	1 6 5 6	4+ 1 1 1	Acid Alk.	1 2 5 6	2 1 1 1	Acid Alk.	0	0	Alk.	Grayish or yellowish	In 4–5 days	Negative	
	22	Well-water	Fleshier	Motile	1 2	2 1	Acid	1 4 bub'le	15 1 —	Alk.	0	0	Alk.	Yellowish	Not coagulated on boiling	Negative	
	23	Well water	Fleshier	Not motile	0 1 5	0	Acid	1 2 1	2 1 —	Acid	0 1	0	Acid	No distinct growth	In 2 days	.....	
	24	Well-water	Fleshier	Motile	1 4 1 5	—	Acid	5 1 6 1		Acid	5 1 6 1	—	Acid	....	In 4–5 days	Positive	
	25	River-water	Fleshier	Motile	1 4	—	Acid	5 1 6	—	Acid	5 1 6	—	Acid	Straw color	Coagulated only on boiling	Negative	

* When two sets of gas-reactions are given, the upper series refers to the temperature of 37° C., the lower to that of 20° to 25° C.

	No.	Source.	Form of surface-colony on gelatine.	Motility	Gas-reaction in fermentation-tube containing one per cent. sugar-bouillon.									Potato.	Milk coagulated.	Indol-reaction.	Remarks.
					Dextrose			Lactose.			Saccharose.						
					Total gas.	H CO_2	Reaction	Total gas.	H CO_2	Reaction	Total gas.	H CO_2	Reaction				
Pseudo-typhoid bacilli.	26	Spring-water	Spreading	Motile	0	0	Acid	0	0	Acid	0	0	Acid	Yellowish	Coagulated only on boiling	Negative	
	27	Well-water	Spreading	Motile	0	0	Acid	0	0	Acid	0	0	Alk.	Yellowish	Coagulated only on boiling	Negative	Growth on potato only at 20° C.
	28	Well-water	Spreading	Motile	0	0	Acid	0	0	Alk.	0	0	Alk.	No distinct growth	No coagulation even on boiling	Negative	
	28*a*	Typhoid	Spreading	Motile	0	0	Acid	0	0	Alk.	0	0	Alk.	No distinct growth	No coogulation even on boiling	Negative	
Bacillus lactis aërogenes group.	29	River-water	Fleshy	Not motile	1 2 1	2 1 3	Acid	1 2 11	2 1 3	Acid	0 2	0 2	Alk.	Yellowish	In 4 days	Positive	Nos. 29–33 inclusive show capsules in the hanging drop (unstained); probably gas-production in solutions containing starch.
	30	Spring-water	Fleshy	Not motile	3 5	1 1	Acid	12 5	2 5	Acid	3 5	1 5	Acid	Whitish, abundant	In 5 days	Negative	
	31	Well-water	Fleshy	Not motile	6 11	1— 4	Acid or alk.	6 2	4	Alk.	6 5	4 5	Alk.	Creamy (gas)	In 1 day	Negative	
	32	Well-water	Fleshy	Not motile	12 1	5 1	Acid	3 3	—	Acid	6 11	4 5	Acid	Creamy (gas)	In 1 day	Negative or very faint	
	33	Well-water	Fleshy	Not motile	1 5	1 1	Acid	4 6	2 5	Alk.	12 1	4 1	Acid	Grayish	In 2–3 days	Negative or very faint	
Bacillus cloacæ.	34	Well-water	Spreading	Motile	6 11	2 1	Alk.	12 5	2 2	Alk.	1 5	2 1	Acid	Yellowish	In 6–7 days	Negative	Nos. 34–36 inclusive liquefy gelatine very slowly.
	35	Well-water	Spreading	Motile	12	2	Acid	12	1	Alk.	6	2	Acid	Yellowish	In 5–6 days	Negative	
	36	Well-water	Spreading	Motile	3/4	7/8	Alk.	3/4	7/8	Alk.	5/6	1—/1	Alk.	Yellowish	In 3 days	Negative	

The method followed by the writer in the general bacteriological examination of water consists, first, in the preparation of gelatine-plates for the usual enumeration; and, second, in the addition to every one of ten fermentation-tubes, containing a 1 per cent. dextrose-bouillon, a certain quantity of water. This is added most easily by first diluting the water, so that 1 or 2 c.cm. are equivalent to the quantity which it is desired to add to each tube. Pipettes graduated by drops are convenient, but not so accurate. In case of ground-water it is well to prepare in addition a flask containing 50 to 100 c.cm. of the water, and an equal, or greater, quantity of bouillon, to which sugar is *not* added. Plates may be prepared from this flask after sixteen to twenty-four hours.* When gas begins to appear in the fermentation-tubes, the amount accumulated at the end of each twenty-four hours should be marked with a glass pencil on the tube. From these tubes, which contain 50 to 60 per cent. of gas on the third day, and are very strongly acid, plates may be prepared to confirm the indications of *B. coli*. This, however, is not essential, for the writer has found as yet no species having these fermentative characters which is not one of the following: *B. coli*, *B. lactis aërogenes*, *B. enteriditis*, *B. typhi murium*, *B. cholerœ suis*. The three last-mentioned species are probably as rare in water as *B. typhosus* itself.

My own experience coincides with that of Mathews when he states that 92 per cent. of all bacteria in ground-water are suppressed in the thermostat. While the addition of 0.5 c.cm., or even more, of such water may fail to produce cloudiness in any of the series of fermentation-tubes, the same quantity, or less, of surface-water never fails to infect the tubes. This difference impressed itself upon me very forcibly in the comparative examination of Potomac water and of some wells in Washington. On one occasion water purporting to come from a well caused clouding of all the fermentation-tubes. This, I knew, was a characteristic of Potomac water in midsummer, when gas-producers are rare. In tracing the matter I found that the person sent to collect the sample had found on the spot designated a public hydrant supplying Potomac water, and had thoughtlessly taken the water from this.

In recommending the use of fermentation-tubes for the detection and isolation of *B. coli* I do not wish to convey the impression that it is to supersede other methods, as, for instance, that of Wurtz. This method is not directly adapted to give us information concerning *B. coli*, nor is it capable of utilizing large quantities of water, excepting by the use of many plates. On the other hand, the method suggested here wholly ignores the search for typhoid bacilli, and focusses our attention upon the strictly fecal species.

* In the case of a certain spring in Washington 50 c.cm. failed to cloud bouillon in the thermostat.

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